REMARKS

Applicant respectfully requests reconsideration of the above-identified patent application in view of the amendment above and the remarks below.

No claims have been canceled or added herein. Claims 1, 2, 6 and 10 have been amended herein. Therefore, claims 1-13 are pending and are under active consideration.

In the outstanding Office Action, the Patent Office states the following sequence listing requirements:

> This application contains sequence disclosures that are encompassed by the definition for nucleotide and/or amino acid sequences set for in 37 CFR 1.82(a)(1) and (a)(2). However, this application fails to comply with the requirement of 37 CFR 1.821 through 1.825 for the reason(s) set forth on the attached Notice To Comply With Requirements For Patent Applications Containing Nucleotides Sequences And/Or Amino Acid Sequence Disclosures. For example, from page 15-18, the specification discloses nucleotide sequences which are not defined by their SEQ ID Numbers.
>
> Applicants are advised that a fully responsive communication **MUST** comply with the Sequence Requirements.

In response to the foregoing requirements, Applicant has amended the specification in this paper to include SEQ ID Numbers for the disclosed sequences. In addition, Applicant is submitting herewith a Sequence Listing on paper, together with a copy of said Sequence Listing in computer readable form and a statement that the Sequence Listing on paper and the Sequence Listing in computer readable form are identical and do not constitute new matter. Accordingly, Applicant is now in compliance with the subject sequence listing requirements.

Claim 6 stands objected to for the following reason:

> Claim 6 recites that a "bisulfate solution" is used as a reagent. However, this appears to be a typographical error since the instant

> specification, on page 9, 2nd paragraph, discloses that the method is conducted with "bisulfite solution." Appropriate correction is required.

In response to the above, Applicant has amended the subject typographical error in claim 6 so that "bisulfite solution" is recited, instead of "bisulfate solution." Accordingly, the foregoing objection has been overcome and should be withdrawn.

Claims 2 and 10 stand rejected under 35 U.S.C. 112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." In support of the rejection, the Patent Office states the following:

> Claim 2 is indefinite for the recitation of the phrase, "whereby the immobilized oligomers hybridize at least to one of the primers or their complementary sequences used in the amplification step," because it is not clear whether the recited complementary sequence is referring to the genomic DNA sequences or other nucleic acid sequences. For the purpose of prosecution, the complementary sequence is assumed to be the genomic DNA sample.
>
> Claim 10 recites the limitation "the primers" and "the hybridizing" in step (b) and (c), respectively. There is insufficient antecedent basis for this limitation in the claim.

Applicant respectfully traverses the foregoing rejection. Claim 2 has been amended herein and now recites "[t]he method according to claim 1, further characterized in that the amplified DNA sample is hybridized to one or more immobilized oligomers, whereby the immobilized oligomers hybridize at least to one of the primers used in the amplification step or sequences complementary thereto in order to achieve the spatial separation." Applicant respectfully submits that claim 2, thus amended, is definite as the recited complementary sequences refer to sequences that are complementary to the primers.

Claim 10 has been amended so that it now depends from claim 2, instead of claim 1. With this change in claim dependency, there is antecedent basis for the language of claim 10.

Accordingly, for at least the above reasons, the foregoing rejection should be withdrawn.

Claims 1-3, 6, 7, 10 and 12 stand rejected under 35 U.S.C. 102(a) "as being anticipated by Gonzalgo et al. (WO 98/56952, published December 17, 1998)." In support of the rejection, the Patent Office states the following:

> Gonzalgo et al. disclose a method of fluorescently detecting the methylated cytosine in a genomic DNA sample (claim limitation 1), wherein the genomic DNA is first treated with a bisulfite (page 4, line 15; claim limitation 6), the DNA amplified by PCR or polymerase chain reaction (page 7-8; claim limitation 7), amplicons separated via electrophoresis (page 5, line 24; claim limitation 3), and the amplicons detected radioactively (page 4, lines 10-30) or fluorescently (page 8, lines 30-31; claim limitation 12). Gonzalgo et al. also disclose a method of detecting the methylated cytosine, wherein the amplicons are transferred onto a nylon membrane for dot-blot analysis (page 8, lines 34-35; claim limitation 2 and 10).
>
> Therefore, Gonzalgo et al. anticipate the invention as claimed.

Applicant respectfully traverses the foregoing rejection. Claim 1, from which claims 2-3, 6, 7, 10 and 12 depend, has been amended herein to more clearly define the subject matter which applicant regards as the invention. As amended, claim 1 recites "[a] method for the relative quantification of the methylation of cytosine bases in DNA samples, characterized in that the following method steps are conducted:

a) a genomic DNA sample is chemically reacted with a reagent, wherein 5-methylcytosine and cytosine react differently and these thus show a different base pairing behavior in the DNA duplex after the reaction;

b) the DNA sample is amplified, wherein a fluorescently labeled dCTP or dGTP derivative is incorporated into the amplified products;

c) the amplified products are separated spatially from each other; and

d) the fluorescence of the separated amplified products is quantitatively measured."

Claim 1 is neither anticipated by nor rendered obvious over Gonzalgo et al. for at least the reason that Gonzalgo et al. fails to teach or to suggest a method for the relative quantification of the methylation of cytosine bases in DNA samples that comprises, among other things, the steps of chemically treating the genomic DNA sample, amplifying the DNA sample, wherein a fluorescently labeled dCTP or dGTP derivative is incorporated into the amplified products, spatially separating the amplified products and quantitatively measuring the fluorescence of the separated amplified products.

Instead, Gonzalgo et al. teaches a method that involves (a) obtaining genomic DNA from a DNA sample to be assayed; (b) reacting the genomic DNA with sodium bisulfite to convert unmethylated cytosine residues to uracil residues while leaving any 5-methylcytosine residues unchanged to provide primers specific for the bisulfite-converted genomic sample for top strand or bottom strand methylation analysis; (c) performing a PCR amplification procedure using the top strand or bottom strand specific primers; (d) isolating the PCR amplification products; (e) performing a primer extension reaction using Ms-SNuPE primers, [^{32}P]dNTPs and *Taq* polymerase, wherein the Ms-SNuPE primers comprise a from about a 15 mer to about a 22 mer length primer that terminates immediately 5' of a single nucleotide to be assayed; and (f) determining the relative amount of allelic expression of CpG methylated sites by measuring the incorporation of different ^{32}P-labeled dNTPs.

Although Gonzalgo et al. does make a passing reference to using "fluorescent probes" instead of a ^{32}P marker, Gonzalgo et al. still falls short of teaching or suggesting the claimed method because, among other things, the Gonzalgo method does not involve amplification of the genomic DNA sample using the fluorescently labeled dinucleotides. Instead, in Gonzalgo et al., the marker is used only **after** amplification of the genomic DNA sample has already been performed; moreover, the incorporation of such a marker requires the use of a methylation specific primer extension reaction, which involves hybridization of a methylation specific primer adjacent to the position to be analyzed. The claimed method does not require such a methylation specific primer extension reaction. Therefore, the claimed method is more simple and, consequently, more time- and cost-effective.

Applicant also wishes to note that, whereas the Gonzalgo method only permits the analysis of single CpG dinucleotide positions, the claimed method permits the quantification of the level of methylation within complete nucleic acid segments.

Therefore, for at least the above reasons, claim 1 is patentable over Gonzalgo et al.

The claims dependent from claim 1 recite additional features and are further patentable over Gonzalgo et al. For example, claim 2 recites the hybridization of the amplificate to an oligonucleotide, the sequence of which is complementary or identical to that of the primers used in the amplification of the nucleic acid. This feature is neither taught nor suggested by Gonzalgo et al.

Accordingly, for at least the above reasons, the foregoing rejection should be withdrawn.

Claims 8, 9 and 13 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Gonzalgo et al. (WO 98/56952, published December 17, 1998) in view of Yurov et al. (Human

Genetics, 1996, vol. 97, pages 390-398). In support of the rejection, the Patent Office states the following:

> Claims are drawn to a method of quantitating the methylation of cytosine bases in a DNA sample which comprises treating the DNA with a reagent, wherein the reagent is a bisulfite, amplifying the treated DNA, and detecting the amplified products via differentially labeled fluorescent dNTPs, wherein the labels are cy3 and cy5.
>
> Gonzalgo et al. disclose a method of fluorescently detecting the methylated cytosine in a genomic DNA sample, wherein the genomic DNA is first treated with a bisulfite (page 4, line 15), the DNA amplified by PCR or polymerase chain reaction (page 7-8), amplicons separated via electrophoresis (page 5, line 24), and the amplicons detected radioactively (page 4, lines 10-30) or fluorescently (page 8, lines 30-31). Gonzalgo et al. also disclose a method of detecting the methylated cytosine, wherein the amplicons are transferred onto a nylon membrane for dot-blot analysis (page 8, lines 34-35).
>
> The method disclosed by Gonzalgo et al. do not employ the differentially labeled fluorescent NTPs comprising cy3 and cy5.
>
> Yurov et al. disclose the use of multicolor fluorescence detection via use of cyanine dyes, more specifically, cy3 and cy4 (page 391, 1st column).
>
> It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the teachings of Gonzalgo et al. with the teachings and suggestions of Yurov et al. to arrive at the invention as claimed for the following reasons.
>
> Although Gonzalgo et al. do not explicitly recite that cy3 and cy5 be used as fluorescent labels, Gonzalgo et al. do suggest that their method is achieved via use of fluorescent labels:
>
> "There are several techniques that are able to determine the relative amount of methylation at each CpG site, for example...or even using fluorescent probes instead of ^{12}P marker" (page 8, lines 30-31).

> In addition to this suggestion, Yurov et al. disclose an explicit benefit of using the cy3 and cy5 dye over the traditional fluorescent labels:
>
> "Cyanine dyes are also useful as fluorescent labels or biological macromolecules. Cyanine 3 dye provides significantly **brighter** fluorescence than any other fluorophore, including fluorescein..." (page 391, 1st column).
>
> Yurov et al. also disclose the advantage of using cy3 and cy5 dyes for multicolor detection (page 391, 2nd column).
>
> Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to take the suggestion of Gonzalgo et al., that is, the feasibility of using fluorescent markers for the detection, with the explicit advantage disclosed by Yurov et al. with a reasonable expectation of success because by doing so, one of ordinary skill in the art would have been able to realize the advantages the cyanine dyes had to offer (i.e., multicolor detection, brighter dyes).
>
> Therefore, the invention as claimed is obvious over the cited references. (Emphasis in original.)

Applicant respectfully traverses the foregoing rejection. Claims 8, 9 and 13 depend from claim 1. Claim 1 is patentable over Gonzalgo et al. for at least the reasons noted above. Yurov et al. fails to cure all of the deficiencies of Gonzalgo et al. with respect to claim 1. Therefore, based at least on their respective dependencies, claims 8, 9 and 13 are patentable over the applied combination of Gonzalgo et al. in view of Yurov et al.

Furthermore, Applicant respectfully submits that there is no basis for combining the references in the manner suggested by the Patent Office.

Accordingly, for at least the above reasons, the foregoing rejection should be withdrawn.

Claims 4 and 5 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Gonzalgo et al. (WO 98/56952, published December 17, 1998) in view of Apffel et al. (U.S. Patent No.

6,379,889 B1, issued April 30, 2002, filed November 4, 1999) and Roche et al. (Biotechnology Progress, 1997, vol. 13, pages 659-668)." In support of the rejection, the Patent Office states the following:

> Claims are drawn to a method of quantitating the methylation of cytosine bases in a DNA sample wherein the separation of the PCR products is achieved by either High Performance Liquid Chromatography (HPLC) or Capillary Gel Electrophoresis (CGE).
>
> Gonzalgo et al. disclose a method of fluorescently detecting the methylated cytosine in a genomic DNA sample, wherein the genomic DNA is first treated with a bisulfite (page 4, line 15), the DNA amplified by PCR or polymerase chain reaction (page 7-8), amplicons separated via conventional electrophoresis (page 5, line 24), and the amplicons detected radioactively (page 4, lines 10-30) or fluorescently (page 8, lines 30-31). Gonzalgo et al. also disclose a method of detecting the methylated cytosine, wherein the amplicons are transferred onto a nylon membrane for dot-blot analysis (page 8, lines 34-35).
>
> The method disclosed by Gonzalgo et al. do not explicitly disclose the use of HPLC or CGE for PCR product separation.
>
> Apffel et al. disclose a method of using HPLC for the separation of PCR amplicons from a PCR reaction mixture (column 3, lines 45-48).
>
> Roche et al. disclose a method of using GCE for the separation of PCR amplicons (pp. 663, 2nd column bottom).
>
> It would have been obvious to one of ordinary skill in the art at the time the invention was made to expand the teachings of Gonzalgo et al. with the teachings of Apffel et al. and Roche et al. to arrive at the invention as claimed per suggestion offered by Gonzalgo et al., wherein the artisans state:
>
> "There are many chromatographic techniques that can be used to isolate PCR amplification products (*or amplicons*)" (pp. 8, line 7-9).

> One of ordinary skill in the art at the time the invention was made would have recognized various chromatographic techniques for separation/purification and the advantage offered by such techniques, as illustrated by Apffel et al. and Roche et al.:
>
> "CE is capable of rapid, automated, reproducible, and high-resolution separation of small volumes of complex mixtures." (pp. 659, 2nd column; pp. 664, 1st column, *Roche*).
>
> "Distinguish individual PCR amplicons (also referred to as PCR products herein) from a PCR reaction mixture." (column 3, lins 44-47).
>
> Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to modify the teaching of Gonzalgo et al. given their explicit statement of feasibility to realize the advantages offered by the separation techniques of Apffel et al. and Roche et al. with a reasonable expectation of success.
>
> Therefore, the invention as claimed is obvious over the cited references.

Applicant respectfully traverses the foregoing rejection. Claims 4 and 5 depend from claim 1. Claim 1 is patentable over Gonzalgo et al. for at least the reasons noted above. Apffel et al. and Roche et al., taken individually or in combination, fail to cure all of the deficiencies of Gonzalgo et al. with respect to claim 1. Therefore, based at least on their respective dependencies, claims 4 and 5 are patentable over the applied combination of Gonzalgo et al. in view of Apffel et al. and Roche et al.

Furthermore, Applicant respectfully submits that there is no basis for combining the references in the manner suggested by the Patent Office.

Accordingly, for at least the above reasons, the foregoing rejection should be withdrawn.

Claim 11 stands rejected under 35 U.S.C. 103(a) "as being unpatentable over Gonzalgo et al. (WO/98/56952, published December 17, 1998) in view of Wang et al. (Science, May 1998, vol. 280, pages 1077-1082)." In support of the rejection, the Patent Office states the following:

> The teachings of Gonzalgo et al. has been set forth above.
>
> Gonzalgo et al. do not explicitly disclose that the amplification was multiplexed.
>
> Wang et al. disclose a method of SNP genotyping which involves multiplex amplification from a genomic DNA via plurality of primers (pp. 1080). Wang et al. multiplexes 46 loci from a genomic DNA (pp. 1080, 3rd column).
>
> It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the teachings of Gonzalgo et al. with the teachings and advantages discloses by Wang et al. to arrive at the invention as claimed for the following reason.
>
> Wang et al. clearly suggest the well-known advantage of multiplexing PCR reactions:
>
> "We next sought to **decrease substantially the sample preparation** required to genotype large numbers of SNPs, as required to perform a genomic scan. We developed a protocol based on multiplex PCR in which primer pairs from many different loci are combined in a single reaction." (page 1080, 3rd column).
>
> One of ordinary skill in the art, therefore, would have been motivated to employ the well-known multiplex-PCR technique into the method disclosed by Gonzalgo et al. for the well-known advantage of reducing the sample preparation/contamination with a reasonable expectation of success.
>
> Therefore, the invention as claimed is obvious over the cited references. (Emphasis in original.)

Applicant respectfully traverses the foregoing rejection. Claim 11 depends from claim 1. Claim 1 is patentable over Gonzalgo et al. for at least the reasons noted above. Wang et al. fails to

cure all of the deficiencies of Gonzalgo et al. with respect to claim 1. Therefore, based at least on its dependency from claim 1, claim 11 is patentable over the applied combination of Gonzalgo et al. in view of Wang et al.

Furthermore, Applicant respectfully submits that there is no basis for combining the references in the manner suggested by the Patent Office.

Accordingly, for at least the above reasons, the foregoing rejection should be withdrawn.

In conclusion, it is respectfully submitted that the present application is now in condition for allowance. Prompt and favorable action is earnestly solicited.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is

required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

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Dated: April 15, 2004

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop Fee Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on April 15, 2004

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